TRANSLATOR'S DECLARATION

Assistant Commissioner for Patents,
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Sir:

I, Nobue Kanaka, declare:

That I am thoroughly familiar with the Japanese and English languages;

That I am competent to serve as a translator of Japanese documents into English;

That the attached document represents a true English translation of Japanese application 2000-208632, filed July 10, 2000 and that

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 26th day of December, 2005.

Nobue Kanaka

Translator

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this office.

Date of Application:	July 10, 2000
Application Number:	Patent Application No. 2000-208632
Applicant:	SECRETARY OF AGENCY OF INDUSTRIAL SCIENCE AND TECHNOLOGY

January 19, 2001

Commissioner,
Patent Office OIKAWA, Kozo (Seal)

Certificate No. 2000-3113408

[Name of document]	Application for patent
[Case number]	117F0110
[Date of filing]	July 10, 2000
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[International patent classification]	C98H 1/00
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[List of submission]		
[Name of submission]	Specification	1
[Name of submission]	Drawing	1
[Name of submission]	Abstract	1
[Proof requested or not]	Yes	

[Document Name] Specification

[Title of the invention] Microsize driving device and preparation method thereof

[Claims]

[Claim 1]

A microsize driving device which comprises: a substrate having a linear track groove; an arrangement of motor protein molecules deposited on the bottom of the linear track groove; and track protein molecules disposed on the arrangement of the motor protein molecules, the linear track groove having side surfaces shaped in a moving direction controlling structure which permits a linear movement of the track protein molecules moving in a specific direction but inhibits the track protein molecules moving in a direction reverse to the said specific direction to cause reversion for the movement in the said specific direction.

[Claim 2]

The microsize driving device as described in claim 1 wherein a part of the side surfaces of the linear track groove is patterned so that a track width is narrowed toward the specific direction and broadened toward the reversed direction.

[Claim 3]

The microsize driving device as described in claim 2 in which the linear track groove has a pattern of which an intermediate position with a part of which one end has a width broader than the lengthwise length of the track protein and the other end has a width narrower than the same.

[Claim 4]

The microsize driving device as described in claim 1 in which the linear track groove has a pattern of which positions of the both ends are not located on a single straight line.

[Claim 5]

The microsize driving device as described in any of claims 1 to 4 in which the bottom of the linear track groove is formed from silicate glass or polystyrene.

[Claim 6]

The microsize driving device as described in any of claims 1 to 5 in which the linear track groove has both side faces made from a melamine-based resin or a (meth)acrylic resin.

[Claim 7]

The microsize driving device as described in any of claims 1 to 6 in which the motor protein is kinesin or myosin.

[Claim 8]

The microsize driving device as described in any of claims 1 to 7 in which the track protein is microtubule or actin.

[Claim 9]

The microsize driving device as described in any of claims 1 to 8 in which the linear track groove has a ring-formed pattern.

[Claim 10]

A method for the preparation of a microsize driving device which comprises the steps of: forming a linear track pattern by providing a photoresist layer on a substrate, which is light-exposed through a photomask followed by development; forming a linear track groove by removing the photoresist layer remaining on the light-exposed areas of the substrate by a plasma treatment or sputtering; forming an arrangement of molecules by pouring a solution containing a motor protein into the linear track groove to deposit molecules of the motor protein onto the bottom thereof; and disposing molecules of a track protein on the molecular arrangement.

[Detailed description of the invention]

[0001]

[Technical field of the invention]

The present invention relates to a novel microsize driving device utilizable for transportation of microsize materials or so as a linear driving device or a rotary driving device within a micrometer-order region as well as to a method for the preparation thereof.

[0002]

[Background art]

A protein which is found in a living body and exhibits mobility function in itself, such as kinesin and myosin, is generally called a motor protein. Kinesin and myosin have an ability to drive fibrous proteins such as microtubules and actin along the fibrous axis thereof by utilizing the energy released when adenosine triphosphate (referred to as ATP hereinafter) is hydrolyzed. These fibrous proteins capable of moving are defined here as a track protein.

[0003]

While kinesin and myosin have a molecular weight of 140 kDa and 500 kDa, respectively, the size of the force generating domains is very small to be 4×5 nm and 5×20 nm, respectively. In microtubules and actin, the fibrous structure is formed by the self-assembly of molecules having a diameter of several nm so that fibers having a length of several tens of μm can be formed by causing self-assembly of these molecules in vitro.

[0004]

As to such motor protein molecules, it is known that movements in random directions are effected when they are adsorbed on the whole surface of a substrate and track protein molecules are disposed thereon and one-dimensionally linear bilateral movements are caused when they are arranged on a linearly patterned layer of a fluorocarbon resin or methacrylic resin and track protein molecules are disposed thereon (see Japanese Journal of Applied Physics, volume 34, 1995, pages 3937-3941; Biophys. J., volume 72, 1997, pages 1997-2001).

[0005]

FIG. 1 is a schematic perspective illustration of a state in which a track 2 formed as a raise on a substrate 1 is provided with an arrangement layer 3 of such motor protein molecules and track protein molecules 4 are disposed further thereon.

[0006]

By the way, if the energy of movement generated between such a motor protein and a track protein could be taken out, the same could be utilized, for example, as a power source for transportation of a microsize body but two problems must be solved therefor.

The first problem is to inhibit disappearance of the track protein molecules disposed on the arrangement of the motor protein molecules arranged within the track on the substrate. Namely, as is shown in FIG. 1, the motor protein molecules are, conventionally, adsorbed on the tracks formed from a fluorocarbon resin or a (meth)acrylic resin, however, these tracks 2 are formed as a raise on the substrate 1 so that the track protein molecules 4 disposed thereon easily fall from the track 2 during movements unavoidably resulting in a decrease of the amount thereof in the lapse of time. Accordingly, it is essential to accomplish an improvement in order to maintain the movement with stability within the tracks over a long time.

[0007]

The second problem is how to control the moving direction of the track protein molecules. When the motor protein molecules are arranged on a linear track and the track protein molecules are disposed thereon by a conventional method, namely, the movement of the track protein molecules is in bilateral directions along the direction of the track so that the kinetic energy of the individual molecules cannot be taken out for utilization as a driving source due to cancellation of kinetic energy of the individual molecules. It is accordingly necessary to control the movement in a single direction in order to accomplish utilization of the kinetic energy as a driving power source.

However, heretofore, absolutely no attempts to solve the above mentioned

two problems for track protein molecules to be moved by motor protein molecules have been made.

[0008]

[Problems to be solved by the invention]

The present invention has been completed with an object to inhibit falling of the track protein molecules from the arrangement of the motor protein molecules on a track provided on a substrate and to enable utilization of the kinetic energy of the track protein molecules as a driving power source by controlling the moving direction thereof.

[0009]

[Means to solve the problem]

The inventors have continued extensive investigations for developing a method to utilize the kinetic energy produced by the arrangement of motor protein molecules and moving track protein molecules disposed thereon and, as a result thereof, have arrived at a discovery that the object can be accomplished by forming the linear track provided on a substrate in a configuration of a groove with deposition of the motor protein molecules on the bottom portion only thereof and by shaping the side surfaces of the groove in such a structure as to permit movement of the track protein molecules moving in a specific direction but to inhibit the track protein molecules moving in a direction reversed thereto causing reversion for the movement into the specific direction leading to completion of the present invention on the base of this discovery.

[0010]

Namely, in the present invention, provided is the microsize driving device comprises: a substrate having a linear track groove; an arrangement of motor protein molecules deposited on the bottom of the linear track groove; and track protein molecules disposed on the arrangement of the motor protein molecules, the linear track groove having a side surface shaped in such a structure as to permit the linear movement of the track protein molecules moving in a specific direction but inhibit the track protein molecules moving in a direction reverse to the specific direction causing reversion for the movement in the above mentioned specific direction and the method for the preparation of the microsize driving device comprises the steps of: forming a pattern of a linear track by providing a photoresist layer on a substrate and exposing the same to light through a photomask followed by development; removing the photoresist layer remaining on the light-exposed areas of the substrate by a plasma treatment or sputtering to form a linear track groove; injecting a liquid containing a

motor protein into the linear track groove to have the motor protein molecules deposited on the bottom thereof forming a molecular arrangement; and disposing track protein molecules on the molecular arrangement.

[0011]

[Preferred embodiment of the invention]

In the following, examples of the embodiments of the present invention are described by making reference to the accompanying drawing.

FIG. 2 is a perspective view schematically showing the structure of the linear track groove in a microsize driving device of the present invention and FIG. 3 is a perspective view of an example in which the linear track groove is formed to have a configuration of the side surface to permit the linear movement of the track protein molecules moving in a specific direction but to inhibit the track protein molecules moving in a direction reverse to the specific direction causing reversion for the movement in the specific direction.

[0012]

In these figures, the motor protein molecules are deposited over the whole surface onto the bottom surfaces of the track grooves 2, 2' provided on the substrate 1 to form molecular arrangements 3, 3' and the track protein molecules 4, ... are disposed thereon. In FIG. 3, the track grooves 2, 2' are provided with wedge-formed notches 5, 5 on both of the respective side surfaces so as to permit movement of the track protein molecules 4, ... in the specific direction (direction A) but to inhibit the movement in the reverse direction (direction B) causing reversion toward the A direction.

[0013]

FIGS. 4(A) and 4(B) are each an explanatory illustration showing the behavior of the track protein molecules in which the side surfaces 6, 6 of the linear track groove 2 are shaped in patterned profile form that the width of the track groove 2 is broadened from right to left or, in other words, narrowed from left to right. While the track protein molecules proceeding from left to right along the arrow mark in (A) can smoothly move along the arrow mark, the track protein molecules proceeding from right to left move along the arrow mark in (B) and hit at a side a to be inhibited from proceeding causing reversion for the movement from left to right.

As a result thereof, the track protein molecules under bilateral movements by means of the motor protein molecules arranged on the bottom surface of the linear track groove 2 enter the movement in a specific direction, namely, in the direction from left to right.

[0014]

FIGS. 5(A) to 5(G) are each a plan view of an example of the pattern profiles 7 provided in the linear track groove shaped in such a fashion that the width of the track is narrowed toward the specific direction and broadened in the reverse direction. The profile is not limited thereto but a great number of modifications besides them are possible. Each of the patterns in FIGS. 5 rectifies the movement of the track proteins to the direction from left to right in the same manner as in the pattern of FIG. 4. As to the dimensions of the pattern there, the rectifying effect on the movement direction can be exhibited with higher efficiency when the width of the entering side of the track protein molecules is larger than the length of the track protein in the lengthwise direction with a narrowed exit opening. In the pattern of FIG. 5(G), the direction along which the track protein molecules enter the rectifying pattern and the direction along which they come out from the pattern are not on the same straight line. In such a case, it is rarely the case that the track protein molecules reversely running from the exit side get out directly from the inlet so as to exhibit a further improved rectifying effect on the moving direction.

[0015]

For the substrate in the microsize driving device of the present invention, metal glass such as silicon, aluminum, tantalum, titanium and the like, for example, silicate glass, fluorocarbon resins such as polytetrafluoroethylene, copolymers of tetrafluoroethylene and hexafluoropropylene, copolymers of tetrafluoroethylene and perfluoro (ethenylalkyl ether), copolymers of poly (monochloro trifluoro ethylene) tetrafluoroethylene and ethylene and the like, acrylic resins such as polymethyl methacrylate, copolymers of methyl acrylate and methyl methacrylate, copolymers of ethyl acrylate and methyl methacrylate and polystyrenes can be used. As the material of the substrate, it is preferable to use one selected from those having affinity with the motor protein molecules to be used and capable of being readily bonded thereto.

[0016]

Next, as the motor protein in the inventive microsize driving device, kinesin, myosin and the like can be used. It is desirable that these proteins are improved beforehand in order to facilitate attaching to the track groove. Such an improvement can be accomplished by the method of, for example, genetic engineering modification of the properties of the motor protein per se or by the method in which the motor protein is biochemically labeled with biotin and attached to the track groove with intervention of streptavidin.

As the track protein used in the inventive microsize driving device, fibrous proteins such as microtubules and actin are used.

[0017]

It is preferable that the linear track groove in the inventive microsize driving device has side surfaces formed of a material to which the motor protein molecules used can attach with difficulty. Such a material includes, for example, melamine-based resins and (meth)acrylic resins.

[0018]

The microsize driving device of the present invention can be advantageously prepared by utilizing the photolithographic technology.

In the following, the preparation method is described by way of an example utilizing silicate glass for the substrate, kinesin as the motor protein and microtubules as the track protein.

That is, a melamine-based or (meth)acrylic photoresist is coated on a silicate glass substrate in a thickness of 1 μm and a pattern of a linear track groove is formed by image-forming light-exposure through a photomask followed by development. In the next place, while it is necessary to bring a kinesin solution into contact with the linear track groove to have the kinesin adsorbed to the glass substrate, mere contacting of the solution is not sufficient for the formation of an arrangement due to random adsorption of the kinesin molecules on either of a glass surface and resin surface.

[0019]

By the way, while adsorption of kinesin on the linear track groove has a bilateral nature of hydrophobic bonding and ionic bonding, resin surfaces are hydrophobic and glass surfaces are ionic so that the difference between these natures can be utilized for the preferential adsorption onto the glass surfaces only. Namely, kinesin molecules can be adsorbed onto the glass surface only by inhibition of adsorption onto the resin surface when a non-ionic surface active agent is added to the kinesin solution to be brought into contact with the glass surface. Preferable non-ionic surface active agents used here include, for example, alkylaryl polyethyleneglycols, polyoxyethylene sorbitan monopalmitates, lauryl alcohol-polyethyleneoxide adducts and the like. Within the linear track groove obtained in this way, the microtubules enter a very stable movement to exhibit a movement constrained to the track over several hours.

[0020]

Other motor proteins, such as, for example, myosin, exhibit different

behaviors to the material of the substrate. It is a possible way in such a case to effect genetic engineering modification so as to change the bonding characteristic to be similar to that of kinesin so that the inventive device can be prepared by the same method even by the use of a motor protein other than kinesin. Moreover, it is not necessary to effect modification of the motor protein when a substrate material having adaptability to the motor protein to be used is selected.

[0021]

Further, in the above described method, the adsorptivity of the motor protein molecules to the substrate can be effectively enhanced by completely removing the photoresist remaining on the substrate after the development treatment. Such a method includes an oxygen plasma etching treatment and a sputtering treatment with an inert gas.

[0022]

As is shown in FIG. 6, the microsize driving device of the present invention is a rotary driving device moved in a single direction by forming the linear track groove in a circular form.

By using the microsize driving device obtained in this way, an ultra fine particle of glass or polystyrene can be transported as bonded to the track protein molecules, and a microsize driving device having a track groove shaped in a circular form is employed, a gear can be rotated by connecting the gear bonded to the track protein molecules onto the circle. Furthermore, a body can be transported in a microsize space as supported on the track proteins by forming two domains in which the track protein molecules are freely movable and connecting them with a linear track capable of rectification therebetween.

[0023]

[Example]

In the following, the present invention is described in more detail by way of Examples.

[0024]

Example 1

A silicate glass plate as the substrate was coated by spin coating with a negative-working photoresist solution (a commercial name "SAL 601", a melamine resin-based photoresist composition produced by Shipley Co.) put thereon in drops to form a coating film having a thickness of 1 μm. After drying, the coating film was light-exposed through a photomask and developed by using MICROPOSIT Developer MF-312 (produced by Shipley Co.) as a developer solution to form a groove-formed

track pattern having a width of 2 μm, length of 500 μm and depth of 1 μm on the substrate surface. After drying of the substrate, a solution prepared by dissolving, in a buffer solution A containing 0.1% of Triton X100 (a commercial name of alkylaryl polyethyleneglycol, Rohm & Haas Co.), 50 mM of potassium acetate, 10 mM of tris acetic acid (pH 7.5), 4 mM of magnesium sulfate, 1 mM of ethyleneglycol bis (2-aminoethyl ether) tetraacetic acid, 7 mM of 2-mercaptoethanol and 25 μg/ml of casein, kinesin in a concentration of 5 μg/ml or 10 μg/ml was put in drops onto the track groove and kept standing at room temperature for 2 minutes in an attempt to deposit the kinesin molecules onto the glass substrate but an arrangement of the kinesin molecules could be obtained in neither of the resin surface and glass surface.

The same procedure as described above was repeated, therefore, except that the glass surface was subjected to an oxygen plasma etching treatment under the conditions of the oxygen flow rate of 150 ml/minute and high frequency voltage of 280 watts for 60 seconds to obtain an arrangement of the kinesin molecules preferentially deposited onto the glass surface only without deposition of kinesin on the resin surface.

A solution of microtubules was put in drops onto the linear track groove obtained in this way to have the microtubules bonded to kinesin followed by the addition of ATP to initiate movement of the microtubules so that the microtubules entered movement along the wall of the track within the track groove and continued a bilateral reciprocating movement with conversion of the direction at a probability of approximately 100% without running off the track.

[0025]

Example 2

A glass substrate was coated by the spin coating method with a methacrylic resin positive type photoresist solution put thereon in drops to give a film thickness of 1 μm and dried at 170 °C for 10 minutes. After a patterning light-exposure to light of 254 nm wavelength through a photomask, development was conducted by using methyl isobutyl ketone. As a result, the light-exposed areas were removed to form a track groove which was similar to Example 1. This substrate was subjected to oxygen plasma etching and a kinesin solution containing a non-ionic surface active agent was put thereon in drops to have the kinesin adsorbed. Kinesin was preferentially adsorbed on the glass surface of the substrate without adsorption onto the resin surface. Microtubules and ATP were added thereto by using the same method as in Example 1 so that the movement of the microtubules could be limited within the track groove and the movement direction was a bilateral movement along

the linear track groove.

[0026]

Example 3

Patterning of kinesin molecules was undertaken in the same manner as in Example 1 except for use of a dual-circular track having a width of 1.5 to 2.5 μm and a radius of 60 μm or 30 μm as a photomask and microtubule molecules were disposed thereon to be put into movement. Thus, the movement of the microtubules could be controlled to a clockwise and counterclockwise rotary movement along the circumference of the track.

[0027]

Example 4

Track grooves having wedge-shaped side faces were formed on the same circular track pattern as in the Example 3 to control the direction of the rotary movement of the microtubules. Patterning of kinesin molecules was undertaken in the same manner as in Example 3 and microtubules were put into movement. The direction of the movement of the microtubules was in perfect coincidence with the direction intended by the rectifying pattern. In this way, the movement of the microtubules could be controlled to be counterclockwise on the larger circumference and, on the other hand, clockwise on the smaller circumference.

[0028]

Example 5

In order to evaluate performance of the rectifying patterns, an attempt of numerical evaluation of the efficiency of the rectifying patterns was made by using a mask shown in Fig. 7. The probability of the cases where the microtubules passed the rectifier without reversion of the direction is examined in the cases where the entering direction of the microtubules toward the rectifier was incorrect direction or the correct direction. The result is shown in the Table 1. In the table, s and t are actual measurement values and shows that a smaller s value means that the movement direction of the microtubules tends to be reversed in the correct direction by the rectifier. As a result, in Pattern C, the s value was 0.59, namely, not so high efficiency was exhibited, however, by changing the shape to that of Fig. 4(A), the s value can be made high efficiency such that 0.27.

[0029]

[Table 1]

	s value	t value
Pattern A	0.27	1.00
Pattern B	0.47	1.00
Pattern C	0.59	1.00

[0030]

Example 6

Microtubules were labeled with biotin by using succinimide labeled with biotin. Polystyrene beads of 1 μm diameter were coated with bovine serum albumin labeled with biotin and further admixed with streptavidin for bonding to the bovine serum albumin labeled with biotin so as to label the surface of the beads with streptavidin. Since streptavidin has four biotin-binding sites per molecule, the surface of the beads was imparted with a possibility of further bonding of biotin. Accordingly, the beads labeled with streptavidin could be bonded to the microtubules labeled with biotin. By causing adsorption of such microtubules onto the substrate having an arrangement of kinesin molecules by using a mask of FIG. 7, the beads could be transported by the microtubules within the pattern.

[0031]

[Effect of the invention]

The present invention is a microsize driving device having new structure not known until now by using an action between motor protein molecules and track protein molecules, and effective as element to carry out movement controlled to microsize of micrometer-level.

[Brief description of the Drawing]

[FIG. 1] is a perspective view schematically showing the performance of a motor protein and a track protein in the prior art.

[FIG. 2] is a perspective view schematically showing the structure of the track groove in the microsize driving device according to the present invention.

[FIG. 3] is a perspective view showing an example of the microsize driving device having a notch in the side surface of a track groove according to the present invention.

[FIG. 4] is an explanatory illustration showing the movement of track protein molecules in the present invention.

[FIG. 5] is a plan view showing an example of the profiles of the side surface of the track groove in the present invention.

[FIG. 6] is a plan view showing an example of the case where the linear track groove in the present invention has a circular ring form.

[FIG. 7] is a plan view of a rectifying pattern used in Example 5.

[Explanation of referenced numerals]

1 substrate

2, 2' linear track grooves

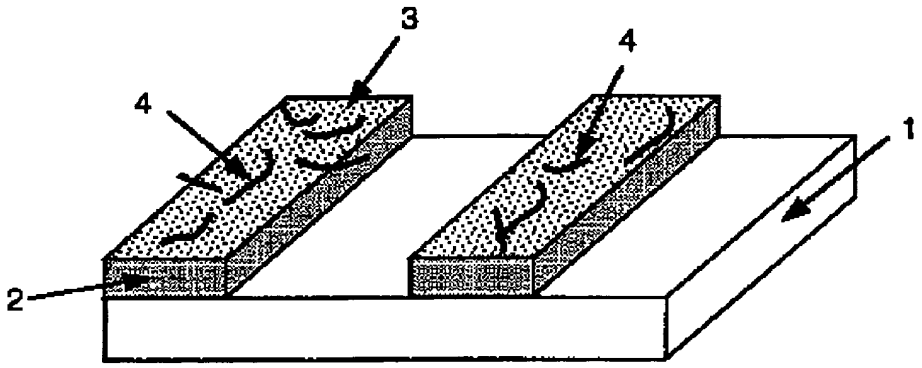
3, 3' motor protein molecular arrangements

4 track protein molecules

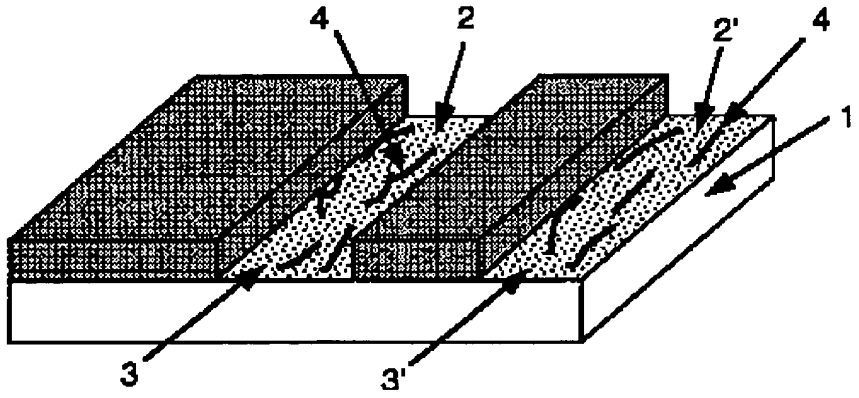
5 notch of track groove side face

[Name of document] Drawing

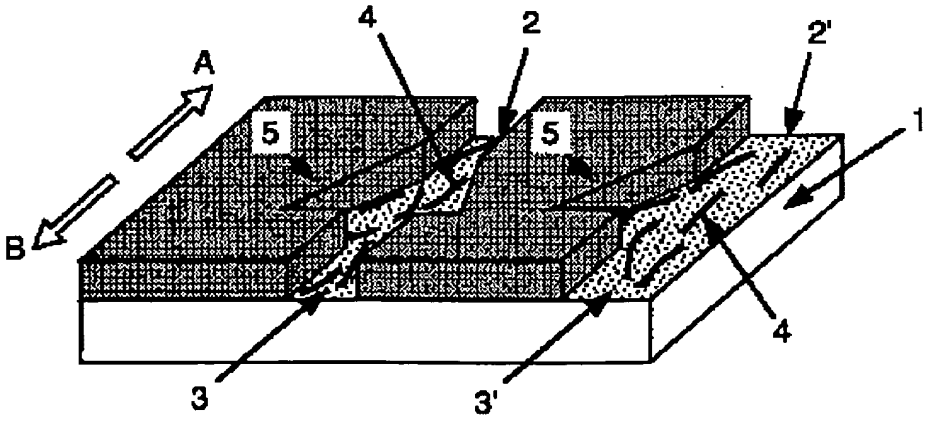
[FIG. 1]



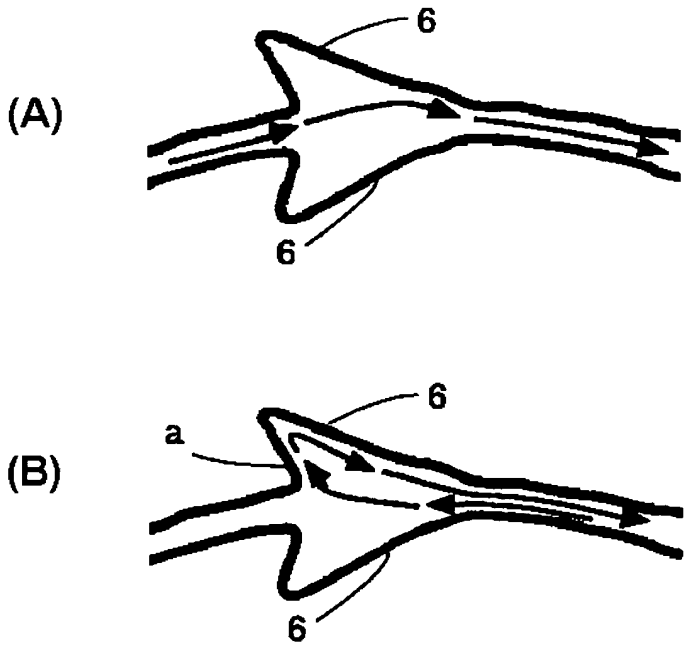
[FIG. 2]



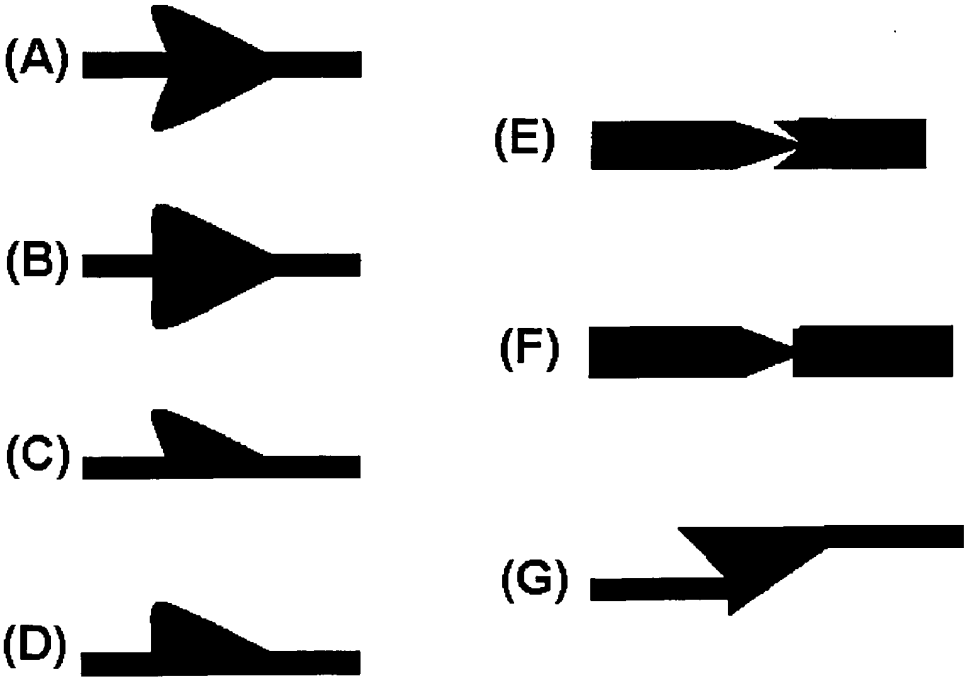
[FIG. 3]



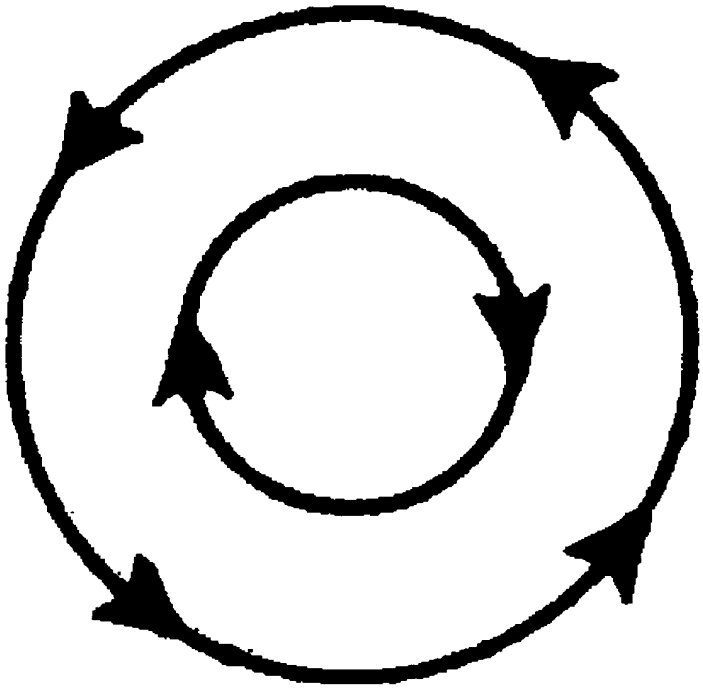
[FIG. 4]



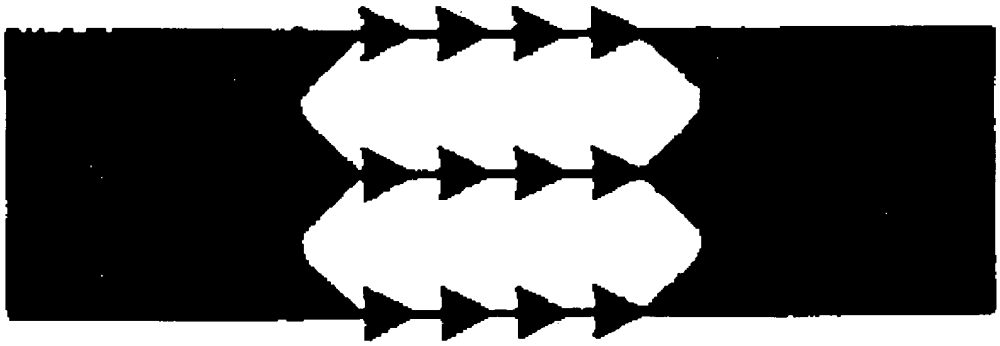
[FIG. 5]



[FIG. 6]



[FIG. 7]



[Name of Document] Abstract

[Abstract]

[Problems to be solved]

Utilization of the kinetic energy of the track protein molecules as a driving source is made possible by suppressing falling of the track protein molecules from the arrangement of the motor protein molecules on a track provided on a substrate and controlling the moving direction thereof.

[Means to solve the problems]

A microsize driving device which comprises: a substrate having a linear track groove; an arrangement of motor protein molecules deposited on the bottom of the linear track groove; and track protein molecules disposed on the arrangement of the motor protein molecules, the linear track groove having side surfaces shaped in a moving direction controlling structure which permits a linear movement of the track protein molecules moving in a specific direction but inhibits the track protein molecules moving in a direction reverse to the specific direction to cause reversion for the movement in the specific direction.

[Selected figure] None

Admitted・added information

Patent application No.	2000-208632
Receipt No.	5000086603
Name of document	Application for Patent
Officer in charge	SATO, Hirosato 7664
Date of preparation	July 24, 2000

<Admitted information・added information>

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INFORMATION OF RECORD OF APPLICANT

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